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Supporting Material

Title: A Predictive Model of Cell Traction Forces Based on Cell Geometry

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Supplemental Figures

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Supplemental Figures

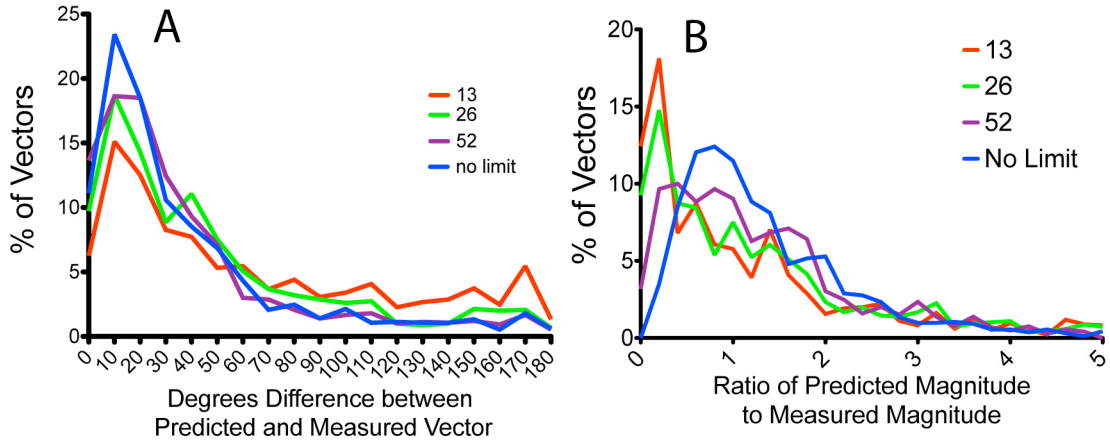

Figure S1. Effects of inter-post distance on truss model performance. "Local" trusses (i.e., truss models in which a given post is only linked to its closest neighbors, instead of to every other post in the cell) were generated, and their performance was compared to the whole cell-truss model. For local trusses, truss elements were only included if they were shorter than a given threshold (legend values, microns). These values correspond roughly to a radius of 1, 2, or 4 posts from a given cell, respectively. The model performs poorly when only nearest neighboring posts are included in the truss (13 micron limit), and improves as longer elements are included. However, none of these local truss models perform as well as the whole-cell truss model. Note: the average cell spans an area of roughly 6 posts by 6 posts. Thus, a radius of 4 posts (which corresponds to the 52 micron local truss model) should encompass the majority of the cell.

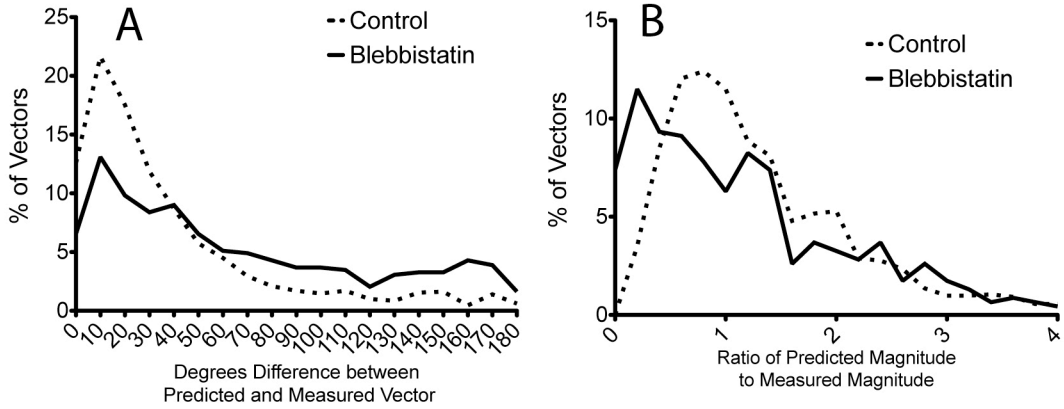

Figure S2. Prediction of forces in blebbistatin-treated cells. Histograms of (a) difference between predicted and measured vector direction (degrees) and (b) ratio of predicted to measured magnitude. Data are shown for control (dashed line) and 20 mM blebbistatin-treated cells (solid line). Data indicate that the FMA/Truss model performs poorly in myosinII-inhibited cells.

Supplemental Methods

Cell Culture and Reagents

Mouse Embryo Fibroblast cells (ATCC) were cultured in DMEM (Sigma, St. Louis, MO) containing 10% fetal bovine serum (Atlantic Biological, Elizabeth City, NC), 1% glutamine, and 1% Penicillin/Streptomycin under standard culture conditions.

mPAD Fabrication

mPADs consist of uniformly spaced grids of deformable silicon posts. Fabrication of mPAD substrates was described previously (1,2) Briefly, an mPAD template was made by pouring PDMS over an array of posts lithographically generated on a silicon wafer from an epoxy-type, near-UV photoresist (SU-8; Shell Chemical, Geismar, LA). The mPAD template was cured overnight at 110°C, peeled from the SU-8 post array, oxidized for 1 min, and treated with (tridecafluoro-1,1,2,2-tetrahydrooctyl)-1-trichlorosilane vapor overnight under vacuum to aid removal of mPADs from the template. mPADs were then made by pouring PDMS onto the template, degassing under vacuum, and curing overnight at 100°C. mPAD post stiffness was measured using calibrated glass needles (World Precision Instruments, Sarasota, FL) on an original mPAD with 11 micron post height and scaled to an appropriate value for 10 micron posts. Surface-oxidized mPADs were then microcontact printed with fibronectin from a PDMS stamp pre-coated with 50 ug/ml human plasma fibronectin (Sigma) to promote cell adhesion to mPAD post top surfaces. mPADs were coated first with 0.5 mg/ml BSA-647 (Invitrogen Molecular Probes, Carlsbad, CA) to facilitate volumetric imaging of the posts, followed by 1% Pluronics F-68 (BASF, Florham Park, New Jersey) to prevent cell adhesion. mPAD posts were 3 microns in diameter, 10 microns tall, and spaced 9 microns apart.

mPAD Immunofluorescence and Data Analysis

MEF Cells were cultured on mPADs for 24 hours, fixed and permeabilized with 3% paraformaldehyde and 0.5% Triton X-100 in PBS, and rinsed with PBS. Blebbistatin-treated cells were allowed 1 hr to attach to mPADS, and were then incubated with 20 mM Blebbistatin for the remaining 24 hours. F-actin was visualized by incubating samples with fluorophore-conjugated phalloidin (Molecular Probes), while mPAD posts were visualized via the fluorescently-tagged BSA that coated the posts. Images were acquired using epifluorescence microscopy (Eclipse 200, Nikon, Melville, NY) with a 60X objective, Openlab software (Improvision, Lexington, MA), and an internally cooled 12-bit CCD camera (CoolSnapHQ, Photometrics, Tucson, AZ). Acquired images were exported as 16-bit TIFF images and read into an original Matlab (The MathWorks, Natick, MA) code written by the authors and designed to analyze mPAD post deflections, as previously described (2,3). Briefly, acquired images were imported, and a thresholding algorithm was used to determine cell area, detect cell edges, and define mPAD post centroids. Deflections were calculated and vector plots of the resulting cell-generated forces were assembled. All analysis was performed using our original code in Matlab on a 2.4 GHz Pentium 4 PC with 2 GB RAM.

Supplemental References

1. Tan, J. L., J. Tien, D. M. Pirone, D. S. Gray, K. Bhadriraju, and C. S. Chen. 2003. Cells lying on a bed of microneedles: an approach to isolate mechanical force. Proc Natl Acad Sci U S A 100:1484-1489.

2. Lemmon, C. A., N. J. Sniadecki, S. A. Ruiz, J. L. Tan, L. H. Romer*, and C. S. Chen*. 2005. Shear force at the cell-matrix interface: enhanced analysis for microfabricated post array detectors. Mech Chem Biosyst 2:1-16. (*these authors contributed equally)

3. Lemmon, C. A., C. S. Chen, and L. H. Romer. 2009. Cell traction forces direct fibronectin matrix assembly. Biophysical Journal. p 729-738.